PLASMA PROGESTERONE AND ESTRADIOL CONCENTRATIONS IN THE BOVINE TREATED WITH PMSG AND $PGF_{2\alpha}$

By

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CHAPTER I

INTRODUCTION

Increasing the incidence of multiple births in beef cattle could markedly improve the reproductive efficiency of cow herds, and calf crops of over 100% could be obtained. The mechanism for doing this has been demonstrated. Numerous researchers have reported that the injection of pregnant mare serum gonadotrophin (PMSG) will induce superovulation and superfetation in cows. However, from a practical standpoint there are several problems limiting the use of this technique.

One of the most important of these problems is the tremendous amount of labor required to carry out the treatments. The occurrence of estrus must be accurately detected to permit rather precise timing of the hormone injections. In addition, because of the variation in time of the occurrence of estrus in a herd of cows, the gonadotrophins must be administered on an individual cow basis.

Another problem associated with the induction of multiple births by gonadotrophin injections is the large variation in the number of ovulations in a group of animals all of which were given the same quantity of hormone. The reasons for this variation have never been determined with certainty. One possibility is that it may be related to the variation in the interval of time between the PMSG injection and estrus. Several studies have shown that as this interval increased, the number of follicles also increased. Reducing the variation in the interval

from PMSG to estrus might also reduce the variation in ovulatory response. The variation in response may also be related to the timing of PMSG injections since it has been reported that a series of two injections, one in the early luteal phase and the other in the late luteal phase of the estrous cycle, tends to reduce the number of excessive ovulations.

In recent years, research with a group of naturally occurring compounds called prostaglandins has suggested a possible means of reducing the labor requirement and controlling the ovulation rate. A single intramuscular injection of prostaglandin $F_{2\alpha}$ into cows with a functional corpus luteum will result in at least 80% of the cows exhibiting estrus in the period two to four days following the injection. With this successful synchronization of estrus, it may be possible to give gonadotrophin injections to cause multiple ovulations at a set interval after synchronization. If all of the cows could be treated on the same day, the labor requirement could be greatly reduced.

In addition, $PGF_{2\alpha}$ has been shown to cause rapid regression of the corpus luteum as evidenced by a rapid decline in plasma progesterone after treatment. Therefore, if $PGF_{2\alpha}$ was given one day after PMSG, it might reduce the number of ovulations by decreasing the interval from PMSG to estrus.

The object of this study was: (1) to determine the superovulatory response of cows to PMSG injections timed from an estrus induced by injecting $PGF_{2\alpha}$; (2) to attempt to control the number of ovulations by reducing the time interval from PMSG to estrus by injecting prostaglandin $F_{2\alpha}$ following the PMSG injection; (3) to determine the superovulatory

response of cows to PMSG and prostaglandin $F_{2\alpha}$ given in mid cycle and in late cycle; (4) to determine the blood plasma concentrations of progesterone and estradiol associated with the above treatments.

CHAPTER II

LITERATURE REVIEW

Plasma Concentrations of Ovarian Hormones During The Bovine Estrous Cycle

The concentrations of progesterone and estradiol in peripheral blood plasma follows a regular pattern through the four periods of the estrous cycle of the bovine: proestrus, estrus, metestrus and diestrus. Proestrus, the period just preceeding estrus, generally lasts two to three days (McDonald, 1971). It is during this time that the ovarian follicles are growing rapidly and the production of estradiol reaches a maximum. Wettemann et al. (1972), Glencross et al. (1973) and Chenault et al. (1975) reported that the plasma concentration of estradiol increases from basal levels of 2-3 pg/ml to a maximum of 6-9 pg/ml. In contrast, Dobson et al. (1973) reported basal levels of estradiol to be 5 pg/ml and a maximum peak of 14 pg/ml. The plasma progesterone concentration decreases rapidly as proestrus advances and declines to about 1 ng/ml or less.

Following proestrus is the period of sexual receptivity, estrus, which lasts approximately 14-18 hours in the cow (McDonald, 1971). Estrus is characterized by high levels of plasma estradiol (6-9 pg/ml), which decline as estrus advances, and a very low plasma progesterone concentration. Plasma progesterone levels during estrus vary between

0 and 1 ng/ml (Kazana and Hansel, 1970; Stabenfeldt et al., 1969; Wettemann et al., 1972; Chenault et al., 1972 and Lemon et al., 1975).

Metestrus, the period following estrus, is characterized by the ovulation of the ripened follicle 12-15 hours after the cessation of estrus (McDonald, 1971). During the first day of metestrus, circulating levels of estradiol return to their basal levels of 2-3 pg/ml and the formation of the corpus luteum begins (Chenault et al., 1975). Plasma progesterone concentrations remain low through most of the 2-3 days of metestrus, reaching their lowest levels approximately twenty hours after the detection of estrus (Sprague et al., 1971). The plasma progesterone concentration begins to increase as the period of diestrus approaches.

Diestrus is by far the longest period of the bovine estrous cycle, lasting approximately 15 days (McDonald, 1971). It is during this time that the corpus luteum develops into a functional body secreting large amounts of progesterone. Plasma progesterone concentrations increase rapidly during the first 5-6 days of diestrus with a more gradual increase to maximum concentrations late in this stage (Stabenfeldt et al., 1969). Plasma progesterone increases from 0-1 ng/ml during estrus to 6-10 ng/ml at its maximum in late diestrus, approximately 4-6 days before the next estrus (Stabenfeldt et al., 1969; Wettemann et al., 1972 and Glencross et al., 1973). During most of diestrus, plasma estradiol remains at basal concerntrations, although some studies have detected increased plasma estrogen for short durations of this period (Glencross et al., 1973; Lemon et al., 1975). During the final few days of diestrus, the concentration of plasma progesterone begins to decrease while plasma estradiol concentrations begin to increase to those observed during proestrus.

Ovarian Response of the Bovine Treated with PMSG

Pregnant mare serum gonadotrophin (PMSG) has been shown to cause superovulation in cows and heifers both before and after puberty. Siedel et al. (1971) demonstrated that prepuberal heifers as young as one month of age could be stimulated to produce large numbers of follicles when given PMSG. Calves of 0, 1, and 2 months of age which were injected with 1500 I.U. PMSG, followed five days later by 50 mg. L.H., produced an average of 0, 9.4, and 28.2 ovulations per group, respectively. Furthermore, by five months of age calves could be stimulated to produce an average of 77.2 ovulations when injected with 1500-2000 I.U. PMSG and 75 mg L.H. five days later. Other research has supported these findings by demonstrating that 17 week old calves given various combinations of PMSG and L.H. or PMSG and H.C.G. produced an average of 53 follicles greater than 1 cm in diameter (Onuma et al., 1970).

The majority of research with PMSG, however, has been concerned with the postpuberal animal. In general, as the dosage of PMSG is increased, the number of ovulations also increased. Gordon et al. (1962) reported that groups of cows given 800, 1000, 1200, 1600 or 2000 I.U. PMSG produced an average of 1.43, 1.77, 2.50, 2.71 and 3.97 ovulations per group, respectively. Rowson (1950) demonstrated that the injection of 3600 or 4500 I.U. PMSG produced an average of 26 follicles and he also reported that when 2000 I.U. chorionic gonadotrophin was injected intravenously after the PMSG, there was an increase in the number of follicles that ovulated.

Differences in the superovulatory response may be due to the method of preparation of PMSG. Rowson (1950) demonstrated that 3600 I.U. of processed PMSG (purified gonadotrophin) produced an average of 13.3 follicles per animal while the same dosage of whole PMSG produced 30.0 follicles per animal. Another report comparing processed PMSG to fresh, whole PMSG and to freeze dried PMSG and obtained 13.9, 20.1 and 14.5 follicles per group, respectively (Brock and Rowson, 1952).

Most researchers working in this area have reported that the major problem with using PMSG is the tremendous variation in ovarian response between animals given the same dosage. Hafez et al. (1963) observed a range in ovulations from 7 to 62 when 3000 I.U. PMSG was given to cows on the sixteenth day of the cycle, followed five days later by 2000 I.U. HCG. He also reported ovulation rates from 6 to 52 when cows were injected with 5000 I.U. PMSG in two equal doses on the sixteenth and seventeenth days of the cycle, plus expressing the corpus luteum and injecting 2000 I.U. HCG. In 1964 Hafez et al. reported a range of 2 to 10 ovulations per cow when treated with 1500 I.U. PMSG on day 16 and a range of 2 to 14 ovulations when 2000 I.U. PMSG was used. These ranges were 2-10 and 2-7, respectively, when 750 I.U. HCG was injected at the first signs of estrus. Scanlon et al. (1968) demonstrated that when cows were given 3000 I.U. PMSG on day 16 of the cycle, followed by 2000 I.U. HCG at the fime of mating, the number of ovulations per cow ranged from 1 to 55. The number of ovulations as a percent of the total follicular response increased from 12.9% to 85.3% as the days from PMSG to estrus increased from 1 to 5.

Schilling and Holm (1963) reported that a series of two injections of gonadotrophin, one early and the other late in the cycle, would tend

to reduce the number of ovulations. They hypothesized that a follicle begins to develop during or just after estrus, and that this follicle grows with others which become atretic. They theorized that an additional dose of PMSG during the early stage of the cycle would either prevent this process of atresia or stimulate the growth of new follicles. They felt that this injection would then produce more follicles ready to develop and that the second injection of PMSG would cause these follicles to grow, instead of many new ones. However, today it is known that only the largest follicle present during the three days before estrus will ovulate.

Hafez et al. (1964) followed this pattern by administering 750 I.U. PMSG on day 5 plus either 1500 I.U. or 2000 I.U. PMSG on day 16 of the estrous cycle. They found that these treatments produced ovulations which ranged from 1-12 and 2-8 for the two groups, respectively.

Laster et al. (1971) obtained a range of 3-6 ovulations per cow when treated with 1500 I.U. PMSG on day 5 and 2000 I.U. PMSG on day 17 of the estrous cycle, followed by 2500 I.U. HCG at the day of estrus. Fifty percent of the cows so treated had 3 ovulations. However, when either 2500 I.U. HCG or 4000 I.U. HCG was administered three days after PMSG, regardless of when estrus occurred, 73.7% of the cows had either two or three ovulations.

Hallford (1975) compared treatments involving either a single injection of 2000 I.U. PMSG on day 17 of the estrous cycle or injections of 1500 I.U. PMSG on day 5 and 2000 I.U. PMSG on day 17 of the estrous cycle. Cows given a single injection had an average ovulation rate of 2.8 as compared to 1.5 for the cows which received two injections. However, 50% of the single injection cows had between two and four

ovulations while only 13.3% of the other cows were within this range of ovulations. When heifers were given a single injection of PMSG, there was an average of 3.1 ovulations with 36.4% of the heifers having between two and four ovulations. With a series of two PMSG injections in heifers, the average ovulation rate was 1.8 and 50% having two to four ovulations. For some unknown reason, heifers and cows responded differently to a single vs. two PMSG injections.

In contrast, Schwartz et al. (1969) showed that a series of two injections in heifers produced more ovulations than a single injection. He observed an average of 6.56 ovulations per heifer with a range of 0-16 in a group which received 1500 I.U. PMSG on day 5 and 2000 I.U. PMSG on day 15 of the estrous cycle, followed by an injection of 1000 I.U. HCG at estrus or day 21. However, in the group of heifers which received 1500 I.U. PMSG on day 15, followed by 1000 I.U. HCG at estrus or day 21, there was an average ovulation rate of 4.72 and a range of 0-13 ovulations. Therefore, although a series of two gonadotrophin injections may tend to reduce the average number of ovulations, it does not appear to be a method for accurate control of ovulation rate.

Effect of PMSG on Ovarian Hormones

The development of methods for quantifying hormone concentrations in the blood has offered a means for studying the mechanism of action of exogenous gonadotrophins. When this mechanism is known, it may be possible to better control the superovulatory response.

PMSG has a dramatic effect on circulating concentrations of progesterone and estradiol. Spillman et al. (1973) reported that when prepuberal heifers 2-5 months old were given 1250-2000 I.U. PMSG

followed in 5 days by 75 mg LH, their plasma progesterone increased from less than 0.5 ng/ml to greater than 100 ng/ml within 10 days after the LH injections. However, none of the follicles ovulated spontaneously, indicating that some of the hormonal relationships between the ovary and the pituitary gland are not well established in the prepuberal animal. When post puberal heifers were injected with 3000 I.U. PMSG on day 15 or 16 of the estrous cycle, followed by 2500 I.U. HCG four days later, the progesterone concentration increased to over 60 ng/ml, which is higher than the 10 ng/ml maximum during the normal estrous cycle.

Hendricks et al. (1973) treated three groups of animals with either 0, 1600 or 3200 I.U. PMSG on day 16 of the estrous cycle. He reported that the mean plasma progesterone concentration began to decrease significantly 60 hours before estrus for the cows given 0 or 1600 I.U. PMSG and about 36 hours before estrus for the cows given 3200 I.U. PMSG. The progesterone concentration decreased to less than 1 ng/ml by 24 hours before estrus in the control cows compared to 12 hours and 6 hours before estrus for the cows given 1600 I.U. and 3200 I.U., respectively. The mean rates of regression of the corpora lutea were significantly steepr for the 3200 I.U. group than for either of the other two groups. This indicated that the plasma progesterone concentration remained higher for a longer period in the cows given 3200 I.U. PMSG, and then decreased at a greater rate once the concentration began to decline.

Hendricks et al. (1973) also studied the plasma concentration of estradiol after PMSG. He reported that the mean rates of increase in estradiol concentration were 0.08, 1.0 and 1.2 pg/ml/hour for the groups

which received o, 1600 and 3200 I.U. PMSG, respectively, when estradiol was measured over the four day period prior to estrus.

Hallford (1975) injected one group of cows with 2000 I.U. PMSG on day 17 of the estrous cycle and another group with 1500 I.U. PMSG on day 5 and 2000 I.U. PMSG on day 17. Plasma progesterone increased and remained between 12 and 16 ng/ml from days 9 to 19 of the cycle for the group which received two injections as compared to values between 6 and 9 ng/ml for the same time period in the cows given PMSG on day 17. When this is compared to a maximum of about 10 ng/ml during the normal estrous cycle, it is evident that an early injection of PMSG in luteotrophic in cows.

Hallford (1975) also reported that plasma estradiol increased from 2 pg/ml to greater than 10 pg/ml after the injection of PMSG on day 5 and decreased to less than 4 pg/ml by day 15 of the cycle. Estradiol increased again after the second PMSG injection on day 17 to a maximum of about 14 pg/ml. When PMSG was only given at day 17, plasma estradiol remained low (2-3 pg/ml) and increased at the time of PMSG injection (day 17) to a maximum of approximately 18 pg/ml. These values are much greater than the maximum concentrations of 6-9 pg/ml during a normal estrous cycle, indicating that PMSG has a dramatic effect on plasma estradiol as well as plasma progesterone.

Response to Repeated Injections of PMSG

Another problem associated with the use of PMSG is the possibility that cows will develop a refractoriness to repeated PMSG injections. A decrease in the response to the gonadotrophin has been reported from several studies in which cows were injected with PMSG on successive

estrous cycles. In 1953 Willet and Buckner reported that by using various concentrations of FSH and PMSG, they obtained an average of 12.9 ovulations per animal after the first injection. This decreased to 3.1 ovulations after the fourth successive injection when the injections ranged from 18 to 218 days apart.

Jainudeen et al. (1966) found no difference in the superovulatory response when they initially injected nine cows with either 1500 I.U. or 2000 I.U. PMSG followed by 3000 I.U. PMSG 7 months later. However, they observed that when cows were treated with 3000 I.U. PMSG during four successive estrous cycles, the ovaries ceased being stimulated. In addition, they reported that the antigonadotrophin in the serum of the treated cows was low prior to the second injection and increased to a maximum 16 days after the fourth PMSG injection.

On the basis of results obtained in a series of experiments, Cole et al. (1957) concluded that antihormone formation was not a serious factor in the clinical use of PMSG if minimal physiological doses were used. No studies have been concerned with the effect of doses of PMSG repeated only at yearly intervals, as in the production of multiple births.

Prostaglandins

Prostaglandins were first reported about forty-five years ago, but they received very little attention until the 1960's. Since that time they have been studied quite extensively and their physiological actions have been shown to be very diverse and to affect many tissues.

In 1930 Kurkrok and Lieb found that human seminal plasma caused contraction of uterine tissue. Goldblatt (1935) and von Euler (1935)

reported that the contractile effect of either human or ram semen on uterine tissue could not be accounted for by any of the known compounds in semen. They hypothesized that this compound came from the prostate gland and gave it the name prostaglandins.

It wasn't until about thirty years later that Berström and his colleagues reported the structure of prostaglandins (Berström et al., 1969). They found that the compounds were highly active, lipid soluble, unsaturated hydroxy acids, all of which contain 20 carbon atoms and had the same basic carbon skeleton, prostanoic acid. The primary prostaglandins are E_1, E_2, E_3, $F_{1\alpha}$, $F_{2\alpha}$, and $F_{3\alpha}$. The E type prostaglandins all contain 11α-hydroxy and 9-keto groups on a five membered ring. The F type prostaglandins are similar to the E compounds except the 9-keto group is reduced to a hydroxyl group. All of the primary prostaglandins contain at least one double bond in their side chains. E_1 and F_1 contain only one double bond. E_2 and F_2 have two double bonds and E_3 and F_3 contain three double bonds. The F prostaglandins can also be either α or β depending on which of the two isomeric alcohols it resembers. The α isomers are the only ones which occur naturally. Therefore, the compounds are named according to their structure, E or F, with a subscript indicating the number of double bonds. Gnerally, the term prostaglandin is reduced to PG and is followed by the letter, subscript number and α or β for any particular isomer, i.e. $PGF_{2\alpha}$.

By 1969, Berström et al. had isolated 14 different naturally occurring prostaglandins from almost every tissue in the body and they hypothesized that these compounds were ubiquitous among mammalian tissue.

In 1969, Pharriss and Wyngarden demonstrated that $PGF_{2\alpha}$ caused a rapid decrease in the progesterone content of ovaries from pseudopregnant

rats. McCraken et al. (1972) performed a series of intricate experiments involving autotransplantation of the ovaries to the neck of sheep. When an ovary with a functional corpus luteum was transplanted to the neck, the corpus luteum did not regress. However, when both the ovary and the uterus were transferred to the neck, the corpus luteum regressed in the normal length of time. This suggested that a factor from the uterus was required to cause luteal regression. They then transplanted an ovary with a corpus luteum to the neck and infused $PGF_{2\alpha}$ into the ovary's blood supply and noted a rapid regression of the corpus luteum. This demonstrated that in sheep, $PFG_{2\alpha}$ was luteolytic, working directly on the ovary and not through the uterus.

Barrett et al. (1971) and Thornburn and Nicol (1971) conducted similar studies which supported McCracken's work. Bland et al. (1971) found that the concentration of $PGF_{2\alpha}$ increased in the uterine vein of sheep on days 14, 15 and 16 of the estrous cycle, but it was not detectable at any other stage. This is direct evidence that $PGF_{2\alpha}$ is the luteolytic factor produced by the uterus of the sheep.

Prostaglandins are now being studied as a potential method for controlling the estrous cycle of cattle. Liehr et al. (1972) observed that injection of 500 ug $PGF_{2\alpha}$ into the ipsilateral horn of the uterus in heifers every hour for six hours did not affect cycle length when administered on day 5 of the estrous cycle. However, when 6 mg $PGF_{2\alpha}$ was injected into the ipsilateral horn on day 9 of the estrous cycle, the animals returned to estrus 2.4 days later and plasma progesterone concentrations decreased to undetectable concentrations within two days after the treatment.

In a similar study, Louis et al. (1972) deposited 5 mg $PGF_{2\alpha}$ in the ipsilateral uterine horn of the uterus of cows on day 11 of the estrous cycle and caused luteal diameter to decrease from 2.7 cm to 0.4 cm by 48 hours later. Estrus occurred an average of 68 hours after treatment with ovulation occurring an average of 94 hours after treatement. Similar results were reported by Rowson et al. (1972). They found that $PGF_{2\alpha}$ depositied in the uterus of cows prior to day 5 of the estrous cycle had no effect on estrous cycle length.

Prostaglandin has also been shown to be effective when administered as a systemic injection. Louis et al. (1973) reported that 30 mg $PGF_{2\alpha}$ in 1.5 ml saline injected IM into heifers during diestrus (8-18 days after estrus) caused plasma progesterone concentrations to decrease from 4.0 ng/ml at the time of treatment to 1.2 ng/ml 72 hours later. Estrus occurred an average of 74 hours after treatment and ovulation occurred an average of 104 hours after treatment. However this study also demonstrated that when $PGF_{2\alpha}$ was administered to heifers during mestestrus (3 days after estrus) normal corpus luteum growth was not affected. In a similar study, Nancarrow et al. (1974) injected cows with either 25 mg or 30 mg $PGF_{2\alpha}$ and normal luteal regression was induced.

These studies established that prostaglandins, specifically $PGF_{2\alpha}$, when administered to a cow with a functional corpus luteum, would cause rapid regression of the corpus luteum, drastically reducing plasma progesterone levels and be followed by the occurrence of estrus about three days later. Therefore, $PGF_{2\alpha}$, could be used to synchronize the estrous cycles of groups of cows.

The fertility of cows at the first estrus after treatment with $PGF_{2\alpha}$ was determined by Lauderdale et al. (1974) with an extensive

experiment at four geographic locations using 392 cattle. The results of their study demonstrated that normal fertility occurred at the synchronized estrus and timed inseminations without estrus detection were possible. Similarly, Hafs et al. (1974) found that fertility in cows treated with $PGF_{2\alpha}$ was not different from that of control cows.

Rowson et al. (1972) reported that when a single systemic injection of $PGF_{2\alpha}$ was administered to cows one day after PMSG, the eggs produced were normal and resulted in normal fertility when transferred to cows which had been synchronized with $PGF_{2\alpha}$. Therefore, because $PGF_{2\alpha}$ has been shown to be luteolytic in cows, and also has no adverse effect on fertility, it may be possible to use this compound with PMSG to reduce the time interval from PMSG treatment to estrus, thereby reducing the number of ovulations.

CHAPTER III

MATERIALS AND METHODS

General

This study was conducted from May 1975 through July 1975. It involved fifty Angus cows and heifers maintained under range conditions on native pasture at the Southwest Livestock and Forage Research Center, El Reno, Oklahoma. The hormone analysis was conducted at the Animal Science Physiology Laboratory on the campus of Oklahoma State University, Stillwater, Oklahoma.

Pregnant Mare Serum Gonadotrophin

The pregnant mare serum gonadotrophin (PMSG) used in this study had been obtained in bulk from Argentina and was stored at -10°C. When originally received it had been standardized to a potency of 200 I.U. per mg. This potency was confirmed just prior to use by the rat bioassay described by Cole and Erway (1941). The test animals used were twenty-one day old immature female albino rats of the Texas Inbred Mice Company, strain Tex:SDD. The standard PMSG was the World Health Organization 2nd International Standard for Serum Gonadotrophin, Equine for Bioassay, provided by the Hampsted Laboratories, London.

The bioassay was conducted with a 3x3 factorial arrangement of treatments with three sources of PMSG (the International Standard plus

PMSG from two separate containers, A&B, sotred in the Animal Science Physiology Lab.) and three dose levels (6, 9 and 13 I.U.) of each source of PMSG. The PMSG was dissolved in sterile saline solution and injected subcutaneously into groups of five rats per PMSG level. The rats were sacrificed 48 hours post injection and the ovaries were removed and weighed. Mean ovarian weights for the five rats in each of the levels tested are in Table I. An analysis of variance (Snedecor and Cochran, 1967) showed no evidence to suggest that either source of PMSG was difference than the International Standard ($P \sim .25$) and the potency was assumed to be 200 I.U. per mg.

Just prior to the bioassay, the PMSG was dissolved in saline solution so that 1 ml of the solution contained 1000 I.U. It was then divided into vials containing 2000 I.U. per vial and frozen and stored at -10°C at the Physiology Lab in El Reno. Just prior to injection the required amount of PMSG was thawed and mixed to a volume of approximately 5 ml per injection. Injections were given subcutaneously in the shoulder region of the animal.

Prostaglandins

The prostaglandin $F_{2\alpha}$-Tham salt used in this study was provided by the Upjohn Company. Prior to the start of the study the prostaglandin was diluted in sterile H_2O at a concentration of 33.5 mg/5 ml which provided a dose level of 25 mg prostaglandin $F_{2\alpha}$, frozen and stored at -10°C until used. The prostaglandin injections were given intramuscularly in the rump of the animal.

TABLE I

MEAN OVARIAN WEIGHTS
FOR PMSG BIOASSAY

Source	Dose		
	6 I.U.	9 I.U.	13 I.U.
Standard	21.67±1.66*	33.80±3.17	39.50±8.27
PMSG A	26.82±1.32	29.76±1.75	38.78±3.58
PMSG B	32.48±5.62	33.96±1.63	48.30±6.96

*Mean ± standard error.

Design

Thirty-four Angus cows and sixteen yearling heifers were used in this study. The animals were observed twice daily for signs of estrus. Estrus detection was facilitated by the use of sterile bulls equipped with chin ball markers. After at least one normal estrous cycle, the animals were randomly assigned within age groups to one of five treatments as described in Table II. All animals, regardless of treatment, were injected intramuscularly with 33.5 mg $PGF_{2\alpha}$ Tham salt between days 7-14 of the estrous cycle. This injection was designated PGF-I. All subsequent prostaglandin treatments were designated PGF-II. Animals on all treatments were bred by natural service at the second estrus after PGF-I.

Treatment I served as the control group. After the PGF-I injection no further treatment was applied. Treatment II received 2000 I.U. PMSG

TABLE II

EXPERIMENTAL DESIGN

Treatment	Time of Injection- Days After PGF-I	
	2000 I.U. PMSG	33.5 mg $PGF_{2\alpha}$ Tham Salt
I	--	--
II	12	13
III	--	13
IV	20	--
V	20	21

subcutaneously on the twelfth day after PGF-I (approximately day 9 of the estrous cycle) followed on the next day by an intramuscular injection of 33.5 mg $PGF_{2\alpha}$ Tham salt. Treatment III did not receive PMSG but was injected with 33.5 mg $PGF_{2\alpha}$ Tham salt thirteen days after PGF-I (approximately day 10 of the estrous cycle).

Treatment IV received 2000 I.U. PMSG twenty days after PGF-I (approximately day 17 of the estrous cycle). Treatment V also received PMSG on day 20 post PGF-I followed by 33.5 mg $PGF_{2\alpha}$ Tham salt the following day (approximately day 18 of the estrous cycle).

Twenty ml blood samples were collected by tail vein puncture into 20 ml evacuated glass containers. Nineteen mg of oxalic acid in 0.3 ml water was added to prevent clotting. The blood was transferred to plastic centrifuge tubes and centrifuged at 2500 RPM for 15 minutes. The plasma was then decanted into plastic vials and stored at -10°C until estradiol and progesterone were quantified.

The blood sampling schedule is presented in Table III. Blood samples were collected from all animals on the day of PGF-I and three days

TABLE III

BLOOD SAMPLING SCHEDULE

Treatment	Day 7-14 of Cycle	Days Post PGF-I													
		3	12	13	14	15	16	17	18	19	20	21	22	23	24
I	PGF-I													bleed daily until estrus or day 27	
	B*	B	B		B		B		B		B		B		
II	PGF-I		PMSG	PGF											
	B	B	B	B	B	B	bleed daily until estrus or day 21								
III	PGF-I			PGF											
	B	B	B	B	B	B	bleed daily until estrus or day 21								
IV	PGF-I										PMSG				
	B	B	B		B		B		B		B	B	bleed daily until estrus or day 27		
V	PGF-I										PMSG	PGF			
	B	B	B		B		B		B		B	B	bleed daily until estrus or day 27		

*B designates days blood samples were taken.

later. In addition, blood samples were taken from treatment I animals every other day during days 12-22 after PGF-I, then daily until estrus or day 27 post PGF-I. Animals on treatments II and III were bled daily until estrus or day 20 starting at day 12 after PGF-I. Animals on treatments IV and V were bled every other day from day 12-20 post PGF-I and then daily until estrus or until day 27 (approximately day 24 of the estrous cycle).

Determination of Ovarian Response

Ovulation rates for treatments II, IV, and V were determined by means of a high lumbar laparotomy between days 7 and 14 days after breeding. Ovulation rates for treatments I and II were determined by rectal palpation.

Animals were held off feed and water overnight before laparotomy. The hair was clipped on the animal's left side between the last rib and the external angle of the ileum, the area cleaned with Roccal-D and thoroughly rinsed with isopropyl alcohol. A total of approximately 20 ml of a two percent procaine solution was injected along both sides of the incision line. An incision of approximately eight inches was made through the skin and outer muscle layer. The inner muscle layer was separated by blunt dissection and a small incision was made into the peritoneum to allow the hand and forearm to enter the body cavity. The ovaries were located and observed through a laporascope (Jacobs-Palmer Operating Peritonescope, Richard Wolf Medical Instrument Corp., 7046 Lyndon Ave., Rosemont, Ill. 60018). The peritoneum and individual muscle layers were sutured separately by means of a continuous stitch. The skin was sutured with umbilical tape. The surgical area was then

painted with iodine and each animal received an injection of 10 ml Combiotic. Skin sutures were removed 10 days to two weeks later. After surgery the animals were returned to pasture equipped with KaMar heat detection patches, where they were exposed to fertile bulls equiped with chin ball markers to aid in determining breeding dates.

Radioimmunoassays

Progesterone

Plasma progesterone was quantified by radioimmunoassay similar to the method of Kittock et al. (1973) as modified by Hallford (1975). One further modification was the elimination of the 5% trimethyl chlorosaline in toluene rinse of the glassware because this did not affect the results of the assay. Standard progesterone was dissolved in freshly distilled ethanol so that 0.1 ml contained 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.6 and 0.8 ng of progesterone. The antibody used was Antiprogesterone #869, generously supplied by Dr. G. D. Niswender, Colorado State University.

Within the assay, samples of water, steer plasma and steer plasma plus 5 ng progesterone were quantified to determine the precision of the assay. When water was used in the assay its average value was 0.1±0.0 ng/ml (mean ± S.E.)(n = 15). The average value for steer plasma was 0.4±0.1 ng/ml (n = 14) and the average value for the steer plus 5 ng progesterone plasma was 5.4±0.1 ng/ml (n = 27), with a between assay coefficient of variation of 13.4%.

Estradiol

Estradiol 17β was quantified by a procedure similar to that described by Wettemann et al. (1972) as modified by Hafs et al. (1974) and Hallford (1975), with one modification. Following the addition of the antibody (Antiestradiol #244) and the estradiol competitor (^{3}H-2,4,6,7 - estradiol) the assay tubes were incubated at 4°C for 4 hours, rather than the 3 to 20 hours as described by Hallford (1975).

Within the assay, samples of water, steer plasma, steer plasma plus 4 pg/ml estradiol, stripped steer plasma, stripped steer plasma plus 5 pg/ml estradiol and stripped steer plasma plus 10 pg/ml were assayed to determine the precision and repeatability of the assay. The stripped steer plasma was produced by adding 5 mg charcoal per ml of steer plasma and stirring for 5 minutes, followed by centrifugation for 20 minutes at 6000 PRM. The above procedure was repeated a second time and the resulting plasma was stored at -10°C until assayed. The average values for the various samples used in this assay were: water, 0.4±0.1 pg/ml estradiol (mean ± S.E.)(n = 31); steer plasma, 4.7±0.3 pg/ml estradiol (n = 15); steer plus 4 pg/ml estradiol, 9.2±1.0 pg/ml (n = 10); stripped steer plasma, 2.2±0.5 pg/ml (n = 6); stripped steer plus 5 pg/ml estradiol, 7.0±0.5 pg/ml (n = 7, c.v. = 18.2%); stripped steer plasma plus 10 pg/ml, 11.1±0.9 pg/ml (n = 5, c.v. = 17.8%). The steer and steer plus 4 pg/ml samples were used during the early part of the study and the stripped steer samples were used during the later part of the study.

During the course of this study, an attempt was made to use a second antibody to separate bound and free estradiol in the assay instead of charcoal and dextran. It was hoped that the double antibody assay

would be more sensitive than the charcoal-dextran assay that was being used.

The first step in developing a double antibody assay was to obtain Rabbit Anti Sheep IgG (Miles-Yeda, Ltd., Lot R167, Code 65-130) to bind and precipitate the Sheep Antiestradiol IgG. This Rabbit Anti Sheep IgG was diluted in Phosphate Buffered Saline plus 0.1% Gelatin (PBS + Gel) and was used at various dilutions to precipitate the Sheep Antiestradiol IgG (diluted with PBS + Gel in 1:100 Normal Rabbit Serum, NRS) and with various concentrations of ^{3}H-2,4,6,7 - estradiol competitor. Once the optimum combinations of diluations had been obtained, several standard curves were assayed to determine the sensitivity in the assay.

Standard estradiol concentrations of 0, 1, 2, 4, 6, 10, 20, 40, 60 and 100 pg in 0.1 ml distilled ethanol were added to assay tubes and dried on a heating block under N_2. Next, 200 ul Antiestradiol #244, at a dilution of 1:100,000 in 1:100 NRS, was added to each tube, vortexed gently and allowed to stand at room temperature. After 2 hours, 200 ul of ^{3}H estradiol competitor containing approximately 500 cpm was added to each tube, vortexed gently and stored in a refrigerator at 4°C for 24 hours. The assay tubes were then removed from the refrigerator and placed in an ice water bath. Then, 400 ul of a 1:90 dilution of Rabbit Anti Sheep IgG was added to each tube, vortexed gently and stored at 4°C for 48 hours. At the end of 48 hours the assay tubes were centrifuged at 3000 RPM for 30 minutes and a 0.5 ml sample was taken from each tube and counted to determine the radioactivity. Two picograms of estradiol was easily distinguished from no hormone with 95% confidence (n=8). Therefore, allowing for procedural losses and for an aliquot to determine recovery, the sensitivity of the assay was about 2.4 pg/ml serum.

Figure 1 depicts standard curves from both the double antibody assay and the charcoal-dextran assay. It had been hoped that the double antibody assay would increase the sensitivity and repeatability of the standard curve in the region from 1 pg/ml to 6 pg/ml. However, the 6 pg/ml standard displaced only 10% of the ^{3}H estradiol as compared to the zero tube for the double antibody assay. This was not as sensitive as the charcoal-dextran assay already in use, in which the 6 ph/ml standard displaced 15-20% of the ^{3}H estradiol as compared to the zero tube. Therefore, the double antibody assay was not used in this study.

Statistical Analysis

Ovulation rates were analyzed by analysis of variance using a completely randomized design (Snedecor and Cochran, 1967). The time from treatment to estrus and endocrine data were analyzed by using the Student's t test (Steel and Torrie, 1960). Simple correlations were used to describe the relationship between maximum plasma estradiol concentrations and ovulation rate.

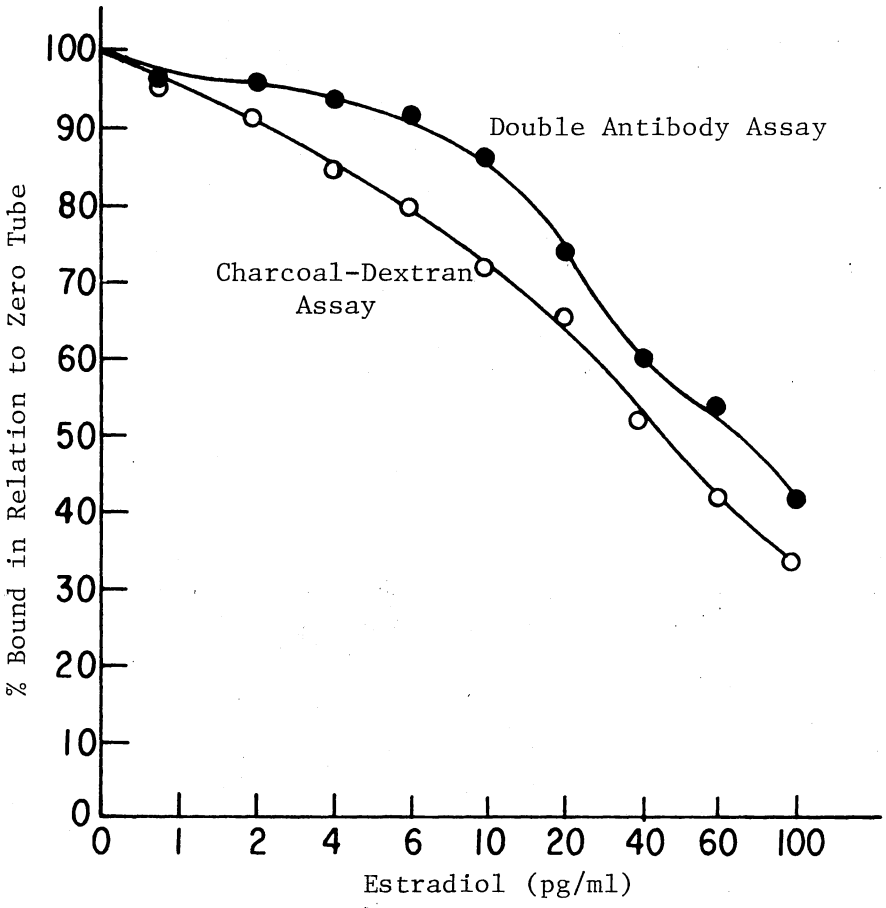

Figure 1. Standard Curves of Double Antibody and Charcoal-Dextran Assays

CHAPTER IV

RESULTS AND DISCUSSION

Reproductive Response

Originally 10 animals were assigned to each treatment on the basis of an observed estrus. However, if the animals were obviously not in the luteal phase of the estrous cycle at the time of PGF-I, or did not respond to the prostaglandin treatment, they were eliminated from the study. The criteria for an animal to be eliminated were if it did not have a normal plasma progesterone concentration at the time of PGF-I injection and/or if plasma progesterone did not decrease to 1 ng/ml by three days after PGF-I. Consequently, the data on only 7 animals in treatments I and II, 9 animals in treatment III, 8 animals in treatment IV and 10 animals in treatment V were used.

Occurence of Estrus After Treatment

Occurence of estrus after treatment is depicted in Table IV. Thirty-six of the 41 animals (87.8%) in this study exhibited estrus following PGF-I. The duration to estrus after PGF-I averaged 3.36±0.21 days for all animals. These results are similar to those reported by Lauderdale et al. (1974) and Nancarrow et al. (1974).

The control animals had an average cycle length of 22.50±0.81 days from the first to the second estrus after PGF-I, with six of seven animals exhibiting a second estrus.

TABLE IV

DAYS TO ESTRUS AFTER TREATMENT

Item	Treatment I	II	III	IV	V
Number	7	7	9	8	10
Estrus Post PGF-I					
Number	7	5	8	6	10
Days	3.6±0.4	3.8±1.0	3.0±0.2	3.3±0.6	3.2±0.2
Estrus After Treatment					
Number	6	5	8	7	10
Days	22.5±0.8[1]	3.0±0.4[2]	3.5±0.3[2]	4.3±0.6[3]	3.8±0.2[3]

[1]Days from 1st and 2nd estrus after PGF-I.

[2]Days from PGF-II to estrus.

[3]Days from PMSG to estrus.

Treatment II animals, which received 2000 I.U. PMSG on day 12 post PGF-I (day 10 of the estrous cycle) and 33.5 mg $PGF_{2\alpha}$Tham salt on day 13 post PGF-I, had an average interval to estrus following PGF-II of 3.0±0.4 days, and 5 to 7 animals exhibited estrus. Treatment III animals, which were given only prostaglandin during mid cycle, had a similar time to estrus of 3.5±0.3 ($P > .30$) and 8 of 9 animals exhibited estrus. These results suggest that PMSG given in mid cycle and followed one day later by $PGF_{2\alpha}$, does not lengthen the time to estrus when compared to an injection of prostaglandin only.

Treatment IV animals which received 2000 I.U. PMSG on day 20 post PGF-I (approximately day 17 of the estrous cycle), were in estrus an average of 4.3±0.6 days after PMSG with 7 of 8 animals exhibiting an estrus. This period from treatment to estrus is similar to the results reported by Hallford (1975). He observed a period of 4.9±0.7 days from treatment to estrus for cows and 4.6±1.1 days for heifers when 2000 I.U. PMSG was administered on day 17 of the estrous cycle. Similarly, treatment V animals, which received the same dose of PMSG on day 20 and 33.5 mg $PGF_{2\alpha}$ on day 21 post PGF-I, were in estrus 3.8±0.2 days ($P > .30$) after PMSG and all 10 animals exhibiting estrus.

Ovulation and Conception Rates

Ovulation and conception rates are presented in Table V. Ovulation rates for treatments II, IV, and V were similar ($P > .50$), being 3.42±1.70, 4.25±1.73 and 3.40±0.91, respectively. The range in the number of ovulations was similar for all groups being 0-13 for treatment II, 0-15 for treatment IV and 0-9 for treatment V.

Table V also presents the percentage of each treatment group which were in each of three ovulation ranges. Brock and Rowson (1952) and Scanlon et al. (1968) reported a decrease in superovulation when the time from treatment to estrus was reduced. It had been hoped that the injection of $PGF_{2\alpha}$ after PMSG would reduce the range in ovulations by reducing the time to estrus so that more animals would have ovulated 2-4 eggs. However, in our study time to estrus was not reduced when PMSG was followed by prostaglandin. Therefore, these figures indicate that the range in ovulations was not reduced to the desirable level. Considering the fact that there were only ten animals on treatment V,

TABLE V

OVULATION AND CONCEPTION RATES

Item	Treatment I Control	II PMSG-Day 12 PGF-Day 13	III PGF-Day 13	IV PMSG-Day 20	V PMSG-Day 20 PGF-Day 21
Number in Estrus	6	5	8	7	10
Ovulation Rate					
Mean	1.0	3.42±0.17	1.0	4.25±1.73	3.40±0.91
Range	---	0-13		0-15	0-9
Conceived to Treatment Estrus	5	3	5	3	3
Open Cows	0	3	2	4	7
Ovulations (%)					
0-1	---	50	---	38	30
2-4	---	33	---	25	40
>4	---	17	---	38	30

and fewer on treatments II and IV, each range in the table contains only 2-4 animals. In order to draw definite conculsions about the effectiveness of $PGF_{2\alpha}$ in controlling ovulation rate after PMSG, more animals must be used.

Pregnancy was disgnosed by rectal polpation 60-90 days after breeding. As can be seen in Table V, conception at first estrus after treatment was quite low. Conception in the four treatment groups averaged 41% as compared to 71% in the control group. Some of the reduction in fertility may be attributed to the laparotomies or rectal polpation, as suggested by Gordon et al. (1962) and Schwartz and Shelly (1969). Heat stress may have been responsible for the reduced fertility. Many of these cows and heifers were bred during July when ambient temperature was elevated. Another possible reason for this reduced fertility could be that only about a 30 day breeding season was permitted after PMSG and cows may not have returned to normal ovarian function by that time. However, it should be noted that 4 animals in treatment IV and 7 animals in treatment V did not conceive to the treatment estrus and did not conceive through the remainder of the 45 day breeding season. In all, 16 of the 34 animals in the four treatment groups (47%) did not conceive during the entire breeding season. Three sets of multiples were born to cows in this study and all three sets were twins. One set of twins was born in each of treatments II (PMSG plus $PGF_{2\alpha}$ in mid cycle), IV (PMSG in late cycle) and V (PMSG plus $PGF_{2\alpha}$ in late cycle).

Progesterone

Plasma progesterone, averaged over all cows in this study, decreased from 7.9±0.4 ng/ml on the day of PGF-I to 0.9±0.1 ng/ml three days later,

which was the approximate day of estrus for most animals. These plasma progesterone concentrations are similar to values for day of estrus reported by Louis et al. (1974), Dobson et al. (1973) and Wettemann et al. (1972). Although these authors reported plasma progesterone concentrations of about 0.5 ng/ml, it should be remembered that the average time to estrus after PGF-I in this study was 3.36±0.21 days. Therefore, some blood samples were collected before the exact day of estrus.

Plasma progesterone had increased to 6.3±0.4 ng/ml by day 12 post PGF-I, which was the first sampling time in the cycle following PGF-I. This value is similar to the luteal phase plasma progesterone concentrations reported by Wettemann et al. (1972) and Glencross et al. (1973). This indicates that the corpora lutea which developed after $PGF_{2\alpha}$ produced normal amounts of progesterone.

Figure 2 depicts the plasma progesterone in treatments I, II and III animals which exhibited estrus following treatment in mid cycle. Plasma progesterone averaged 7.7±1.8 ng/ml, 9.8±1.1 ng/ml and 7.7±0.6 ng/ml for treatments I, II and III, respectively, on the day that PGF-I was given. These values are similar for all three groups ($P > .30$). By three days later, these concentrations had decreased to similar values of 0.8±0.0 ng/ml, 0.9±0.1 ng/ml and 0.9±0.1 ng/ml ($P > .20$).

By nine days later, (day 12 post PGF-I) plasma progesterone had increased to concentrations normally found during the estrus cycle. However, the animals in treatment III had a significantly higher ($P \approx .05$) concentration that treatment II animals on day 12 - 8.2±1.1 ng/ml vs. 4.9±0.8 ng/ml. The only explanation which can be proposed is that this difference was due to chance.

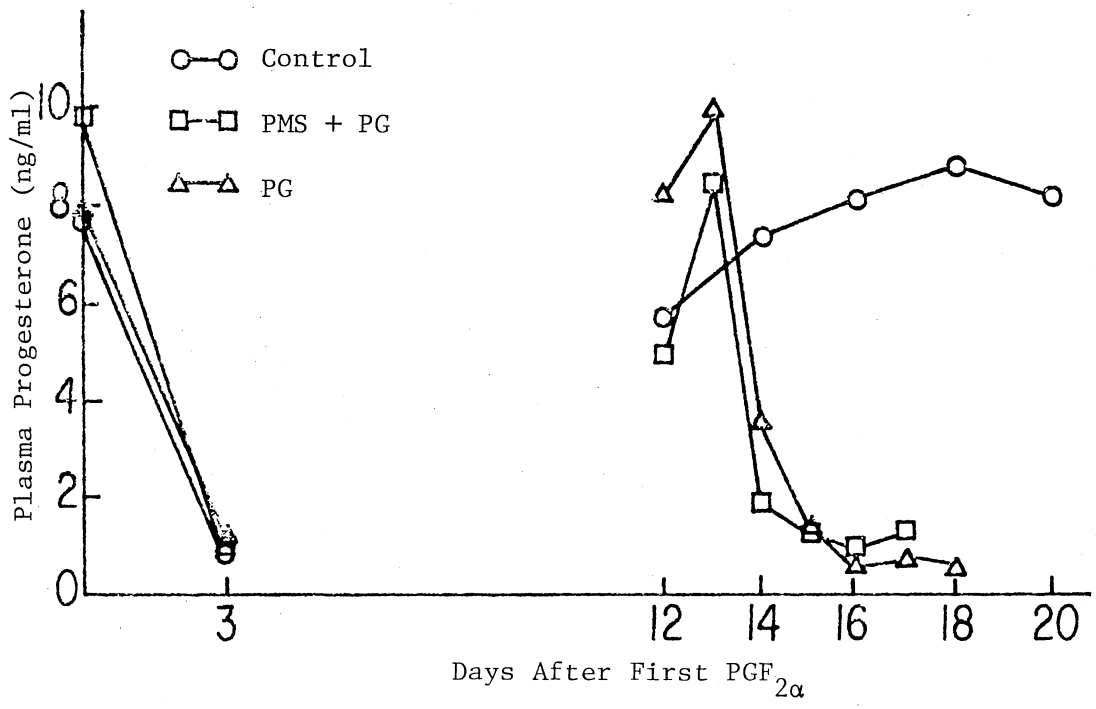

Figure 2. Plasma Progesterone In Animals Exhibiting Estrus Following Treatment at Mid Cycle

On day 12 post PGF-I, animals in treatment II were injected with 2000 I.U. PMSG. On day 13 post PGF-I, plasma progesterone in treatment II had increased to 8.5±2.0 ng/ml which was not significantly different from the 10.0±1.2 ng/ml found in treatment III animals ($P > .40$). On day 13 post PGF-I both treatments II and III received 33.5 mg $PGF_{2\alpha}$ Tham salt. Plasma progesterone decreased rapidly and similarly in both treatments II and III to 1.9±0.3 ng/ml and 3.6±0.6 ng/ml by 24 hours after the $PGF_{2\alpha}$ injection, and 1.0±0.1 ng/ml and 0.7±0.1 ng/ml by 72 hours after the injection of prostaglandin for treatments II and III, respectively. These results are similar to those reported by Louis et al. (1974) and Nancarrow et al. (1974) for progesterone values 72 hours after $PGF_{2\alpha}$ injection. Control animals exhibited normal luteal growth during this period. The plasma progesterone concentrations in the control groups for days 12, 14 and 16 post PGF-I were 5.8±0.5, 7.4±1.1 and 8.2±1.3 ng/ml.

Of the 2 cows which did not exhibit estrus in treatment II (PMSG plus $PGF_{2\alpha}$ in mid cycle), one had plasma progesterone concentrations similar to other cows on that treatment. However, the other heifer apparently did not respond to the $PGF_{2\alpha}$ injection given after PMSG. The plasma progesterone concentration for this animal continued to increase and reached a maximum of 18.24 ng/ml 7 days after the PMSG injection.

Only one heifer on treatment III ($PGF_{2\alpha}$ in mid cycle) did not exhibit estrus following treatment. The plasma progesterone concentration in this animal was 11.4 ng/ml on day 13 post PGF-I when the second prostaglandin injection was administered. Progesterone decreased to 6.6 ng/ml one day after PGF-II and to a minimum value of 3.1 ng/ml 3 days

after prostaglandin treatment, at which time it started increasing again. The reason for this response is not clear.

These results indicate that PMSG given at mid cycle does not significantly increase plasma progesterone when prostaglandin is given the next day. In addition, the luteal regression after $PGF_{2\alpha}$ does not appear to be altered when PMSG is given the previous day.

Plasma progesterone in animals exhibiting estrus after treatment in late cycle is depicted in Figure 3. Progesterone was similar for all three treatments between days 12-20 post PGF-I increasing from 5.8±0.5 to 8.2±1.7 ng/ml for the control; from 6.5±0.8 ng/ml to 7.99±0.79 ng/ml for treatment IV; from 6.6±0.9 ng/ml to 10.20±0.7 ng/ml for treatment V.

Following the injection of treatments IV and V with 2000 I.U. PMSG on day 20 post PGF-I, plasma progesterone increased 12.6±0.8 ng/ml in treatment IV and to 12.1±0.6 ng/ml in treatment V within one day. These values could not be tested against the progresterone for the control group because no blood sample was taken from this group on day 21 post PGF-I.

Treatment V animals received 33.5 mg $PGF_{2\alpha}$ Tham salt the day after PMSG, or approximately day 18 of the estrous cycle. Following this injection plasma progesterone decreased from 12.1±0.6 ng/ml on the day of treatment to 2.6±0.4 ng/ml one day later. This value was significantly less than the 10.8±2.3 ng/ml found in the control animals ($P < .05$). Plasma progesterone had decreased to 1.5±0.4 ng/ml by 2 days after the prostaglandin injection in treatment V and this was lower than the 8.5±1.5 ng/ml in treatment IV animals ($P < .01$) and lower that the 5.0±1.5 ng/ml found in the control animals ($P \sim .05$). By three days

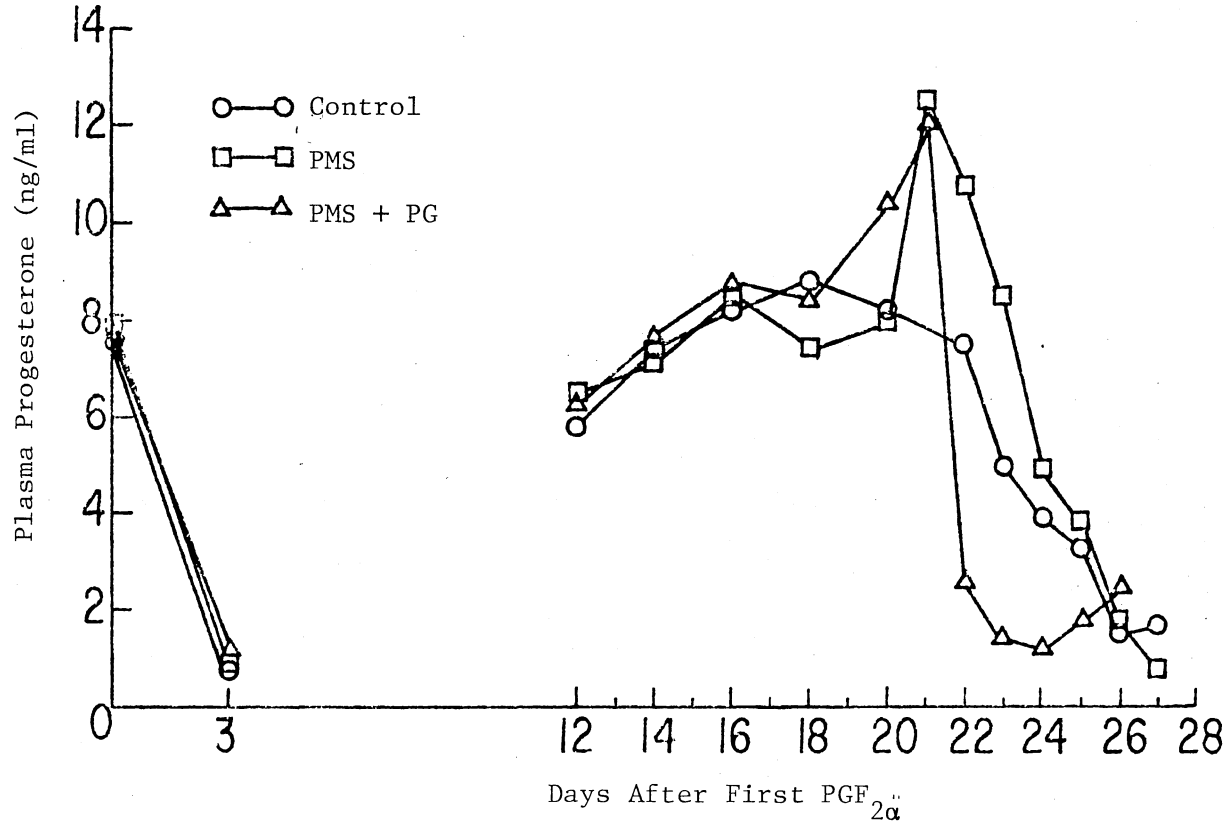

Figure 3. Plasma Progesterone in Animals Exhibiting Estrus Following Treatment Late in the Cycle

after the prostaglandin treatment, or on approximately day 21 of the estrous cycle, plasma progesterone had declined to 1.2±0.5 ng/ml in treatment V. This was not different than the 4.9±1.9 ng/ml found in treatment IV ($P > .10$) or the 3.9±1.9 ng/ml found in the control animals ($P > .10$). Throughout this time period of days 22-24 post PGF-I, the plasma progesterone in treatment IV was never significantly elevated over the progesterone found in the control animals ($P > .20$).

One cow in treatment IV did not exhibit estrus following treatment. Plasma progesterone in this animal increased from 12.8 ng/ml on the day of the PMSG injection to a maximum of 16.2 ng/ml 3 days later, which was approximately day 20 of the estrous cycle. Plasma progesterone began to decrease rapidly and was 0.8 ng/ml on day 27 post PGF-I (day 24 of the estrous cycle) when blood sampling was terminated. It is possible that if the observation continued another one or two days, this animal would have been detected in estrus.

These results indicate that when $PGF_{2\alpha}$ is administered one day after PMSG in late cycle, plasma progesterone is significantly reduced during the two days after prostaglandin compared to when PMSG is given alone. In addition, PMSG plus prostaglandin causes plasma progesterone to be significantly reduced when compared to the untreated controls over the two day period following the prostaglandin injection.

PMSG plus $PGF_{2\alpha}$ was administered both in mid and late cycle (treatments II and V). The injection of PMSG was followed one day later by a plasma progesterone concentration of 8.5±2.0 ng/ml in treatment II which was similar to the 12.1±0.6 ng/ml for treatment V ($P > .10$). $PGF_{2\alpha}$ was given to animals in each group on the day after PMSG. By one day after $PGF_{2\alpha}$, plasma progesterone averaged 1.9±0.3 ng/ml for treatment II and

2.6±0.4 ng.ml in treatment V ($P > .10$). This similar and rapid decline in plasma progesterone continued and on the third day after $PGF_{2\alpha}$, plasma progesterone averaged 1.0±0.1 ng/ml in treatment II and 1.2±0.5 ng/ml in treatment V. This would indicate that the injection of PMSG, followed one day later by $PGF_{2\alpha}$, illicts a similar response in plasma progesterone when this treatment is given either during mid estrous cycle or late in the cycle.

Estradiol

When averaged over all animals in this study, plasma estradiol increased from 3.7±0.3 pg/ml on the day of PGF-I to 7.2±0.6 pg/ml 3 days later, which was the average day of estrus for most animals. These concentrations are similar to those reported by Wettemann et al. (1972) and Chenault et al. (1975).

Plasma estradiol had decreased to 4.3±0.3 pg/ml by day 12 post PGF-I, which was the next sampling time in the cycle following PGF-I. This is slightly higher than the basal estradiol levels during the bovine estrous cycle reported by Wettemann et al. (1972) and Hallford (1975), but is similar to the basal estradiol concentrations reported by Dobson et al. (1974). This return to basal concentrations of plasma estradiol indicates that plasma estradiol levels are normal in the cycle following an estrus induced by $PGF_{2\alpha}$.

Figure 4 depicts plasma estradiol in the animals on treatments II and III which exhibited estrus following treatment during mid cycle. Estradiol concentrations for the control animals (treatment I) are included for comparison. Control animals had an average estradiol concentration of 2.5±0.4 pg/ml on the day of PGF-I as compared to the

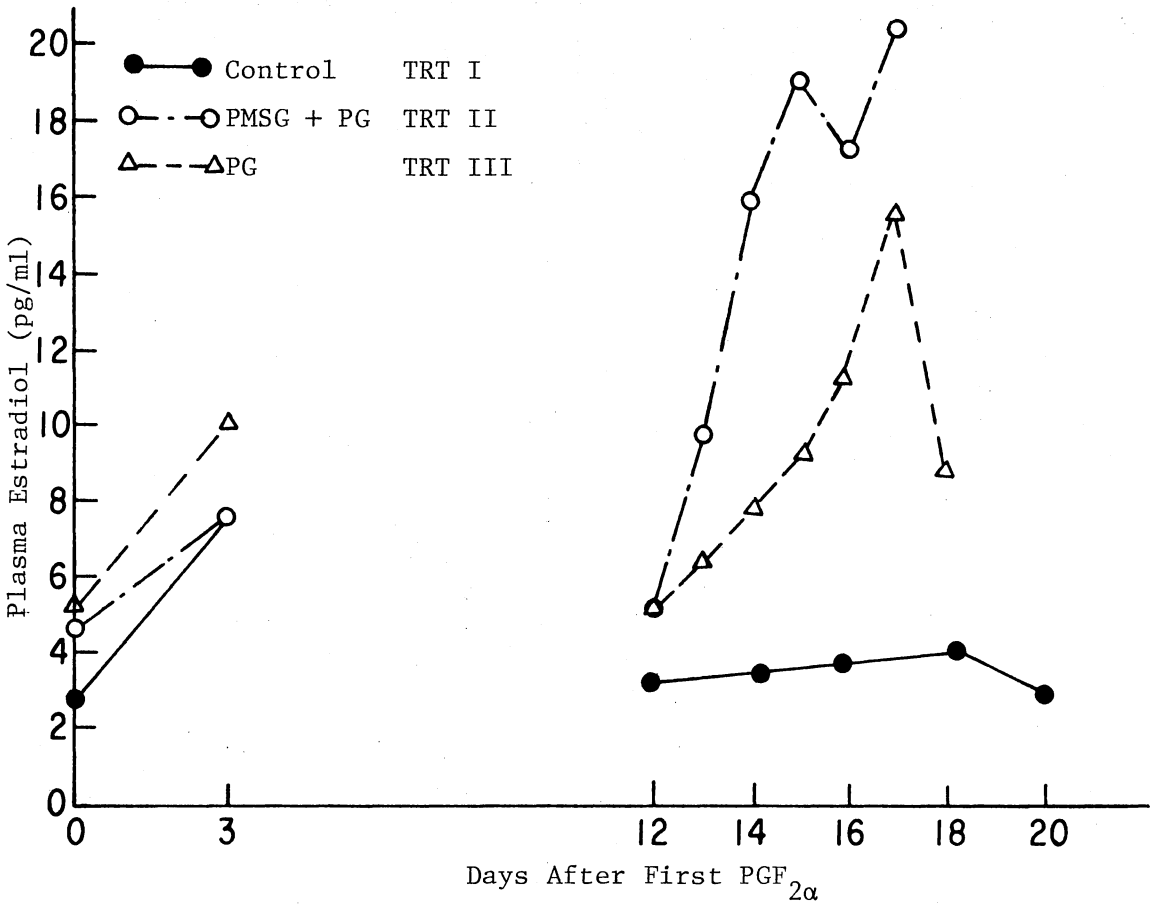

Figure 4. Plasma Estradiol in Animals Exhibiting Estrus Following Treatment At Mid Cycle

4.7±0.2 pg/ml found in treatment II and the 4.9±0.7 pg/ml found in treatment III. This treatment I value is less than both the treatment II ($P < .10$) and treatment III ($P < .05$) values. This is probably due to an inherent difference in the basal estradiol levels between the treatment groups. Despite the differences in the initial concentrations, plasma estradiol increased at about the same rate for the three groups and by three days after PGF-I had increased to 7.5±0.8 pg/ml, 7.4±0.9 pg/ml and 9.9±2.2 pg/ml for treatments I, II and III, respectively.

By day 12 post PGF-I, treatment II animals averaged 5.5±0.2 pg/ml plasma estradiol and treatment III averaged 5.3±0.7 pg/ml. These values were greater than the 3.2±0.5 pg/ml found in the treatment I animals on this same day ($P < .10$). This is probably related to the differences in basal estradiol levels which was noted on the day of PGF-I.

On day 12 post PGF-I, animals in treatment II were injected with 2000 I.U. PMSG. By one day later, plasma estradiol had increased to 9.8±2.8 pg/ml. However, there was little evidence to suggest that this value was different than the 6.8±0.8 pg/ml found in treatment III ($P > .20$), probably as a result of the large range in estradiol levels found in treatment II (2.0 pg/ml - 16.0 pg/ml).

On day 13 post PGF-I, both treatments II and III received 33.5 mg $PGF_{2\alpha}$Tham salt (PGF-II). One day later, plasma estradiol averaged 16.0±5.5 pg/ml for treatment II, 7.7±1.4 pg/ml for treatment III and 3.4±0.3 pg/ml for the control animals. This value for treatment II was not significantly different than the treatment III value ($P > .20$). However, the treatment II value was significantly greater than the treatment I value ($P < .05$), and the treatment III value approached being different than that of the control ($P \sim .10$).

The rapid increase and large variation in plasma estradiol following PMSG and $PGF_{2\alpha}$ in treatment II can probably be attributed to two causes. Injection of PMSG causes rapid follicular growth (Hallford, 1975; Schwartz and Shelly, 1969; Rowson, 1950), and accompanying this follicular growth an increase in plasma estradiol can also be expected (McDonald, 1971). Other work has shown that $PGF_{2\alpha}$ causes rapid regression of the corpus luteum and a rapid decrease in plasma progesterone. This was confirmed in this study, as discussed in the earlier progesterone section. The combination of large numbers of follicles and rapid follicular growth caused by the PMSG, and the rapid decline in plasma progesterone caused by the prostaglandin, probably account for the rapid increase in plasma estradiol found in treatment II.

Although plasma estradiol in treatment III does not reach the same high levels found in treatment II, a rapid increase in estradiol can be seen after PGF-I. This is probably due to the rapid decrease in plasma progesterone as a result of luteal regression following $PGF_{2\alpha}$, which allows the follicles to grow rapidly, secreting large amounts of estradiol.

By two days after PGF-II, plasma estradiol had increased to 19.0±8.7 pg/ml in treatment II and to 9.0±1.0 pg/ml in treatment III. Again, there was no evidence to suggest a significant statistical difference between these two means ($P > .20$) due to the tremendous range found in treatment II (5.2 pg/ml - 52.6 pg/ml).

Day three post PGF-II was the average day of estrus for treatments II and III. The high estradiol concentration found in treatment II (PMSG plus $PGF_{2\alpha}$), 17.2±2.7 pg/ml, is probably due to the large number of growing follicles, rapid follicular growth and the rapid progesterone

decline. The treatment II value is greater than the 11.7±1.1 pg/ml found in treatment III ($P < .10$) and the 3.9±0.9 pg/ml found in the controls ($P < .01$). The value for treatment III was also greater than the controls ($P < .01$).

This treatment III value is somewhat higher than the 6-9 pg/ml estradiol at estrus reported by Wettemann et al. (1972) and Chenault et al. (1975). These studies reported estradiol values at estrus for a normal cycle. The fact that the estradiol values found in treatment III are from an induced estrus in mid cycle may account for the elevated levels. Another point to consider is that the basal estradiol level in treatment III was significantly elevated over the control at both the time of PGF-I and day 12 post PGF-I. Therefore, this elevated estradiol at the average day of estrus might also be due to the overall higher estradiol concentrations found in treatment III.

By day 4 post PGF-II, all but two animals in both treatments II and III had exhibited estrus. The average estradiol concentration for the 2 animals in treatment II was 20.5±0.4 pg/ml. This value was similar to the 15.7±2.4 pg/ml found in the 2 animals which had not yet exhibited estrus in treatment III ($P > .20$).

Estradiol concentrations on the actual day of estrus of each animal averaged 22.6±8.0 pg/ml for treatment II (PMSG plus $PGF_{2\alpha}$) and 12.4±1.4 pg/ml for treatment III ($PGF_{2\alpha}$). However, there was no evidence to suggest a significant difference between these two groups ($P > .30$). The larger estradiol concentration for the actual day of estrus in treatment II can probably be attributed to the large number of ovulations found in this group. Treatment II had an average of 3.4±0.2 ovulations per animal. The average plasma estradiol concentration for control animals

at the day of estrus was 7.0±0.6 pg/ml. Therefore, it is possible that animals having more than 3 ovulations could produce more than 20 pg/ml estradiol on the day of estrus.

The 12.4±1.4 pg/ml estradiol found in treatment III on the day of estrus is higher than those values reported for estrus during the normal estrous cycle by Wettemann et al. (1972) and Chenault et al. (1975). However, this is probably due to animal variation combined with the small number of animals in this treatment.

The correlation between peak estradiol values and the number of ovulations in treatment II was .92 ($P < .05$). There was one animal in treatment II which had plasma estradiol concentrations of 35.0 pg/ml on day 1 post PGF-II and 56.6 pg/ml on day 2 post PGF-II. This animal also had a total of 13 ovulations and this was probably the cause of such high estradiol concentrations. Had this animal not bee included in the calculations, the variation in treatment II would have been greatly reduced.

There were two animals in treatment II (PMSG plus $PGF_{2\alpha}$) which did not exhibit an estrus following treatment. Both of these animals had plasma estradiol concentrations greater than 20 pg/ml from days 2-6 post PGF-II. One of these animals had rapidly declining plasma progesterone concentrations similar to the remainder of the treatment group, but the other had consistently high plasma progesterone from days 3-6 post PGF-II (6.9 ng/ml - 18.2 ng/ml). However, neither of these animals had multiple ovulations. Therefore, the response in these animals suggests that the very high levels of estradiol and/or progesterone, and the absence of an observed estrus cannot be attributed entirely to super-ovulation.

There was one animal in treatment III ($PGF_{2\alpha}$) which did not exhibit an estrus following treatment. The plasma estradiol in this animal followed the same pattern as the remainder of the group, reaching a maximum of 9.9 pg/ml on day 5 post PGF-II.

It should be noted that the control animals exhibited normal plasma estradiol concentrations during the sampling period, days 12, 14, 16 and 18 post PGF-I, ranging from 3.5±0.5 pg/ml to 4.0±0.6 pg/ml.

These results indicate that the injection of 2000 I.U. PMSG, followed one day later by 33.5 mg $PGF_{2\alpha}$Tham salt, in mid cycle causes a rapid increase in plasma estradiol. This treatment elevates plasma estradiol over those concentrations found in the control animals by the first day after prostaglandin treatment. There was a trend for plasma estradiol after PMSG plus prostaglandin in mid cycle to be greater than when prostaglandin is given alone in mid cycle, although this difference was not statistically different except for the third day after prostaglandin treatment. Plasma estradiol on the day of estrus for treatment II was not significantly different than that found in treatment III. The correlation coefficient between peak plasma estradiol and number of ovulations following PMSG and $PGF_{2\alpha}$ in mid cycle was .92, indicating a high correlation between these two parameters.

Plasma estradiol in animals exhibiting estrus following treatment in late cycle is depicted in Figure 5. The initial injection of prostaglandin caused plasma estradiol concentrations to increase from 3.7±0.3 pg/ml to 7.2±0.6 pg/ml by three days later. The increase in plasma estradiol in treatment IV is not as great as the increase found in treatments I and V. However, the rapid decrease in plasma progesterone discussed earlier for this group is evidence of a true response

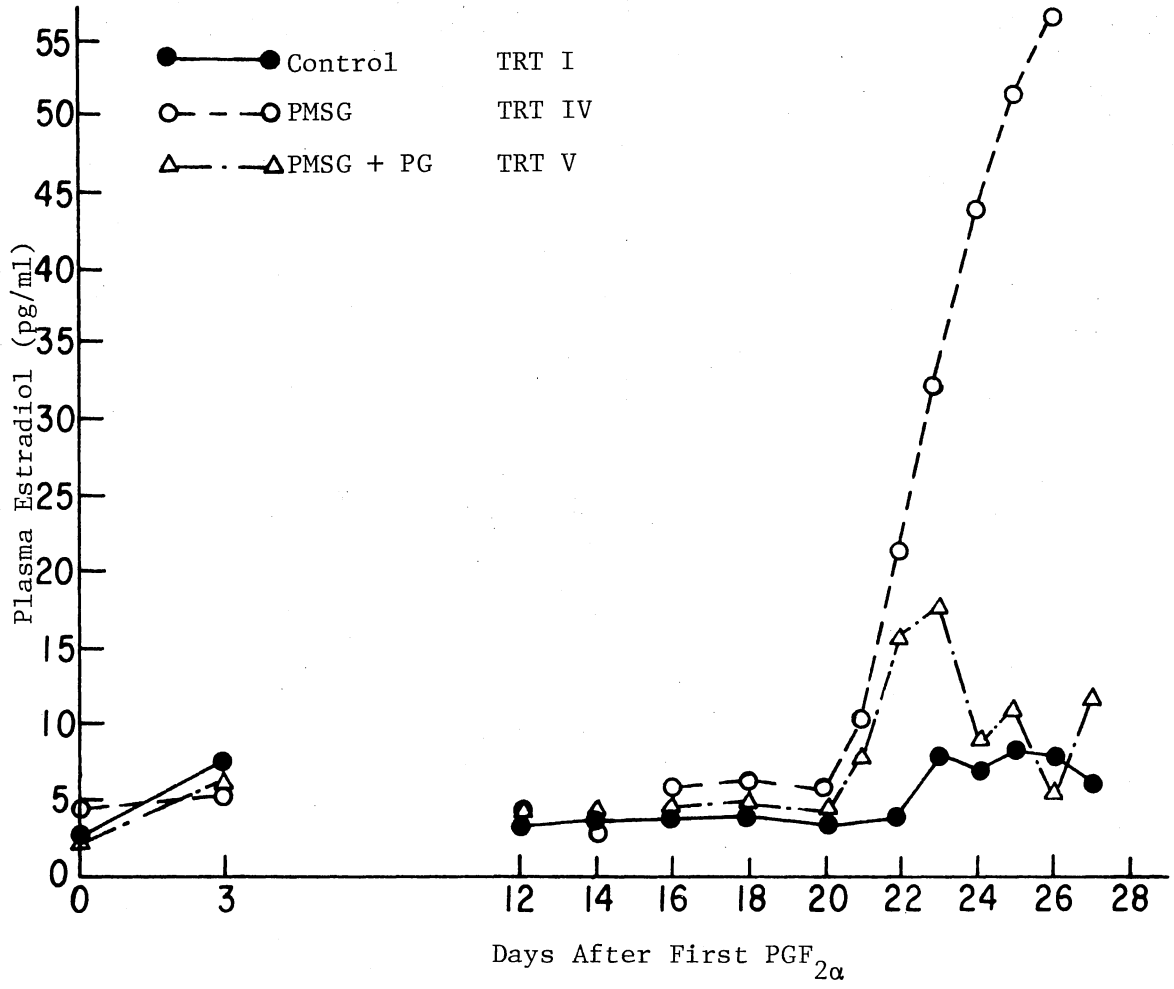

Figure 5. Plasma Estradiol in Animals Exhibiting Estrus Following Treatment Late in the Cycle

to the prostaglandin injection. The most logical explanation for the day 3 post PGF-I estradiol value being low is that the maximum may have occurred the day before or after sampling.

By day 12 post PGF-I, plasma estradiol had decreased to 3.1±0.5 pg/ml for the control group, 3.9±1.0 pg/ml for treatment IV and 3.5±0.6 pg/ml for treatment V. Plasma estradiol remained low between days 12-20 post PGF-I for all three groups, varying between 3.2±0.5 pg/ml and 6.2±0.9 pg/ml for treatment IV; between 3.5±0.6 pg/ml and 4.4±0.7 pg/ml for treatment V and between 3.1±0.5 pg/ml and 4.0±0.6 pg/ml for the control animals. Some of these basal estradiol levels may appear quite high. In some instances, as few as four values comprise a daily mean, because of missing samples. Glencross et al. (1973) and Lemon et al. (1975) have reported that some animals will exhibit a small estradiol peak during diestrus. In this study, several animals in treatments IV and V had a daily estradiol concentration of 8 pg/ml once or twice during diestrus. On some sampling days, more than one animal within a treatment group had increases in estradiol which increased the average estradiol concentration for that day.

Following the injection of 2000 I.U. PMSG on day 20 post PGF-I in treatments IV and V, plasma estradiol had increased one day later to 9.3±2.7 pg/ml in treatment IV and to 8.0±0.9 pg/ml in treatment V ($P > .50$). These values are similar to those reported by Hallford (1975) for one day after the injection of PMSG in late cycle. This rapid increase in plasma estradiol is probably the result of rapid follicular growth caused by the PMSG.

On day 21 post PGF-I, or approximately day 17 of the estrous cycle, treatment V received an injection of 33.5 mg $PGF_{2\alpha}$ Tham salt. Plasma

estradiol on day 22 post PGF-I averaged 22.1±10.2 pg/ml for treatment IV. There was no evidence to suggest a difference between this value and 15.9±6.8 pg/ml for treatment V ($P > .50$), or the 3.9±0.4 pg/ml found in the control animals ($P > .10$). The trememdous range in estradiol values found in treatment IV on day 22 post PGF-I (6.0 pg/ml - 83.7 pg/ml) produces a very large variance which accounts for the fact that the estradiol value for this treatment is not significantly higher than that for the control animals. The rapid increase in plasma estradiol found in treatment V is probably due to the combination of rapid follicular growth caused by the PMSG, and the rapidly declining plasma progesterone following the prostaglandin injection.

By day 23 post PGF-I, or approximately day 20 of the estrus cycle, plasma estradiol had increased to 32.7±9.0 pg/ml for treatment IV (PMSG in late cycle) which was significantly greater than the 7.9±1.2 pg/ml found in the control animals. The plasma estradiol concentration in treatment IV was not significantly different from the 17.6±5.5 pg/ml observed in treatment V, although there was a trend for the treatment IV estradiol to be greater than treatment V ($P \sim .10$). However, on day 24 post PGF-I, the 8.7±2.5 pg/ml found in treatment V was significantly less than the 43.8±16.6 pg/ml estradiol found in treatment IV ($P < .10$).

Treatment V (PMSG plus $PGF_{2\alpha}$ in late cycle) exhibited rapid luteal regression as evidenced by plasma progesterone decline. Although the time to estrus was not significantly different between these two groups (4.3±0.6 days vs. 3.8±0.2 days for treatment V) there was a trend for the prostaglandin group to have a shorter time to estrus. These differences are probably due to the $PGF_{2\alpha}$ given to treatment V. This shorter

time to estrus would allow less time for the follicles to grow, thereby secreting less estradiol prior to estrus.

The 6.9±1.6 pg/ml of plasma estradiol found in the control animals on day 24 post PGF-I was significantly less than the 43.8±16.6 pg/ml found in treatment IV ($P < .10$).

By day 25 post PGF-I, or approximately day 22 of the estrous cycle, there were only two animals which had not exhibited an estrus in treatment IV. Plasma estradiol averaged 51.3±33.7 pg/ml for these animals. On this same day there were also two animals in treatment V which had not exhibited estrus and these averaged 11.0±2.4 pg/ml estradiol. Five of the six control animals had not exhibited estrus by day 25 post PGF-I and there averaged 7.2±2.4 pg/ml estradiol.

The estradiol concentrations for the day of estrus in treatment IV averaged 41.2±4.8 pg/ml. This was significantly higher than the 15.7± 5.9 pg/ml found on the day of estrus for treatment V ($P < .05$) and also higher than the 7.0±0.6 pg/ml in the control group ($P < .001$).

The very high estradiol concentration on the day of estrus in treatment IV can probably be attributed to two factors. This group has an average of 4.2±1.7 ovulations per animal. This number of growing follicles, combined with the longer time that they had to grow and secrete estradiol prior to estrus (4.3±0.6 days) is probably the reason that plasma estradiol was so great.

The estradiol concentration on the day of estrus in treatment V (PMSG plus $PGF_{2\alpha}$ in late cycle) was also elevated above the normal estrus value of 6-9 pg/ml estradiol. This is probably due to the fact that this group had an average of 3.4±0.9 ovulations per animal. The reason that the estradiol concentration at estrus in treatment V is less

than that found in treatment IV is probably that the time to estrus following treatment was slightly less for treatment V, allowing less time for follicle growth and estradiol secretion.

The 7.0±0.6 pg/ml for the day of estrus in the control animals agrees with those values reported by Wettemann et al. (1972), Chenault et al. (1975) and Glencross et al. (1974). Correlations between maximum estradiol concentrations and the number of ovulations were .71 ($P < .10$) for treatment IV and .84 ($P < .005$) for treatment V. This would indicate that maximum plasma estradiol values following PMSG and/or $PGF_{2\alpha}$ in late cycle is correlated to ovulation rate.

There was one animal in treatment IV which did not exhibit estrus following treatment. The plasma estradiol in that animal followed the same pattern displayed by the others on treatment IV, reaching a maximum of 23.1 pg/ml on day 24 post PGF-I.

There was one animal in the control groups which did not exhibit a second estrus after PGF-I. The plasma estradiol in this animal followed the same pattern as the other controls reaching a maximum of 9.4 pg/ml on day 26 post PGF-I, or approximately day 23 of the estrous cycle. This would indicate that these animals either exhibited an estrus which was not detected, or had a silent estrus.

PMSG plus $PGF_{2\alpha}$ was administered during mid estrous cycle and late in the cycle (treatments II and V). The injection of PMSG was followed one day later by a plasma estradiol concentration of 9.8±2.8 pg/ml for treatment II which was similar to the 8.0±0.9 pg/ml found for treatment V ($P > .50$). $PGF_{2\alpha}$ was given to each of these treatments on the day after PMSG. By one day after $PGF_{2\alpha}$, plasma estradiol averaged 16.0±5.5 pg/ml for treatment II and 15.9±6.8 pg/ml in treatment V ($P > .50$). At

two days after $PGF_{2\alpha}$, animals on treatment II averaged 19.0±8.7 pg/ml estradiol and treatment V averaged 17.6±5.5 pg/ml estradiol. This would indicate that estradiol response after PMSG plus $PGF_{2\alpha}$ given during mid estrous cycle is similar to the response found after this same treatment in late cycle.

These results indicate that an injection of PMSG in late cycle will cause plasma estradiol to be elevated over the untreated controls by three days after the PMSG injection. PMSG given alone in late cycle did not signifiantly increase plasma estradiol over PMSG plus prostaglandin given in late cycle until the fourth day after the PMSG treatment, although a trend toward greater estradiol in treatment IV was evident by the third day after PMSG. One reason why differences in plasma estradiol between treatments IV and V were not detected earlier could be due to the tremendous variation in plasma estradiol after the PMSG injections. Treatment IV animals (PMSG in late cycle) had maximum plasma estradiol concentrations ranging from 19.8 pg/ml to 100 pg/ml while the treatment V animals (PMSG plus $PGF_{2\alpha}$ in late cycle) had maximum estradiol concentrations ranging from 13.2 pg/ml to 69.0 pg/ml. Plasma estradiol on the day of estrus was elevated when PMSG was given along in late cycle, compared to either untreated controls or PMSG plus $PGF_{2\alpha}$ in late cycle. In addition, maximum plasma estradiol and the number of ovulations are correlated following treatment with PMSG and $PGF_{2\alpha}$ in late cycle.

CHAPTER V

SUMMARY

The objectives of this study were: (1) to determine the superovulatory response of cows to PMSG injections timed from an estrus induced by injecting $PGF_{2\alpha}$; (2) to attempt to control the number of ovulations after PMSG by reducing the time from PMSG to estrus by injection of $PGF_{2\alpha}$ following the PMSG injection; (3) to determine the superovulatory response of cows to PMSG and $PGF_{2\alpha}$ given in mid cycle and in late cycle; (4) to determine the blood plasma concentrations of progesterone and estradiol associated with these treatments.

Thirty-one Angus cows and ten yearling Angus heifers were used in this study. After exhibiting at least one normal estrous cycle, all animals were given an injection of 33.5 mg $PGF_{2\alpha}$Tham salt (designated PGF-I) between days 7-14 of the estrous cycle and assigned to one of five treatment groups: I. control, no further treatment after PGF-I; II. 2000 I.U. PMSG on day 12 post PGF-I and 33.5 mg $PGF_{2\alpha}$ on day 13 post PGF-I; III. 33.5 mg $PGF_{2\alpha}$Tham salt on day 13 post PGF-I; IV. 2000 I.U. PMSG on day 20 post PGF-I; V. 2000 I.U. PMSG on day 20 post PGF-I and 33.5 mg $PGF_{2\alpha}$ on day 21 post PGF-I. All animals were bred by natural service at the second estrus after PGF-I. Blood samples were collected at various intervals throughout the treatment period via tail vein puncture.

Thirty-six of the 41 animals (87.8%) exhibited estrus following PGF-I with an average time to estrus 3.4±0.2 days post injection. The control animals had an average cycle length of 22.5±0.8 days with 6 of 7 animals exhibiting a second estrus. Treatment II animals (PMSG plus $PGF_{2\alpha}$ in mid cycle) averaged 3.0±0.4 days from the prostaglandin treatment to estrus with 5 of 7 animals exhibiting estrus. The average time from prostaglandin treatment to estrus in treatment III ($PGF_{2\alpha}$ in mid cycle) was 3.5±0.3 days with 8 of 9 animals exhibiting estrus. This was similar to the time to estrus found in treatment II ($P > .30$). A similar time to estrus ($P > .30$) after the PMSG injection was also found between the 4.3±0.6 days for treatment IV (PMSG in late cycle) and the 3.8±0.2 days found in treatment V (PMSG plus $PGF_{2\alpha}$ in late cycle). Seven of the 8 animals in treatment IV exhibited estrus and all 10 animals in treatment V exhibited estrus.

Ovulation rates for the animals receiving PMSG, treatments II, IV and V, were 3.4±1.7, 4.2±1.7, and 3.4±0.9, respectively. There was no difference between ovulation responses ($P > .50$). Conception to the treatment estrus for the four treatment groups averaged 41% as compared to the 71% found in the control group. In all, 47% of the animals in the four treatment groups did not conceive during the entire breeding season.

After initial injection of prostaglandin plasma progesterone decreased from 7.9±0.4 ng/ml on the day of PGF-I to 0.9±0.1 ng/ml three days later. The injection of PMSG plus $PGF_{2\alpha}$ in mid cycle did not increase plasma progesterone over the untreated controls. The combination of PMSG and $PGF_{2\alpha}$ in mid cycle showed the same rate of plasma progesterone decrease as did $PGF_{2\alpha}$ alone in mid cycle. Plasma progesterone

decreased from 8.5±2.0 ng/ml on the day of the second prostaglandin injection to 1.0±0.1 ng/ml three days later for treatment II and from 10.1± 1.2 ng/ml to 0.7±0.1 ng/ml over the same period for treatment III.

Following the injection of PMSG in late cycle (day 20 post PGF-I), plasma progesterone increased one day later from 8.0±0.8 ng/ml to 12.6± 0.8 ng/ml in treatment IV and from 10.2±0.7 ng/ml to 12.1±0.6 ng/ml in treatment V. Injection of cows in treatment V with $PGF_{2\alpha}$ on day 21 post PGF-I was followed by significantly lower ($P < .05$) plasma progesterone within one day compared to levels in cows given PMSG along in late cycle. In addition, PMSG plus prostaglandin in late cycle caused plasma progesterone to be significantly reduced when compared to the untreated controls for the two day period after the prostaglandin treatment. The injection of PMSG alone in late cycle tended to increase plasma progesterone over the untreated controls, although this difference was not significant at any time after treatment.

Plasma estradiol, averaged over all cows in this study, increased from 3.7±0.3 pg/ml on the day of PGF-I to 7.2±0.6 pg/ml three days later. The injection of PMSG in mid cycle, followed one day later by $PGF_{2\alpha}$ (treatment II), was followed by increased plasma estradiol over the untreated controls by the first day after the prostaglandin treatment. This treatment did not significantly increase plasma estradiol over those concentrations found when only $PGF_{2\alpha}$ was given in mid cycle (treatment III), except for the third day after the prostaglandin treatment. However, there was a trend toward higher plasma estradiol in treatment II. The very large variation in the estradiol response to PMSG in treatment II probably resulted in there being no evidence to suggest a difference between plasma estradiol concentrations in treatments II and III. Plasma

estradiol averaged 22.6±8.0 pg/ml on the day of estrus for treatment II and was 12.4±1.4 pg/ml on the same day for treatment III ($P > .30$). The correlation coefficient between peak plasma estradiol and the number of ovulations in treatment II was .92 ($P < .05$).

The injection of PMSG in late cycle (treatment IV) significantly elevated plasma estradiol over the untreated controls by the third day after PMSG. However, PMSG alone in late cycle did not raise plasma estradiol over those values for PMSG plus $PGF_{2\alpha}$ given in late cycle until the fourth day after PMSG, although there was a trend ($P \sim .10$) toward higher plasma estradiol in treatment IV by the third day after PMSG. The plasma estradiol for the day of estrus after treatment averaged 41.2±4.8 pg/ml for treatment IV (PMSG in late cycle). This was significantly higher than the 15.7±5.9 pg/ml found at estrus for treatment V ($P < .05$) and also higher than the 7.0±0.6 pg/ml found in the control group ($P < .001$). The correlation coefficient for peak plasma estradiol and ovulation number was .71 ($P < .10$) for treatment IV and .84 ($P < .005$) for treatment V. The endocrine response after treatment with PMSG and $PGF_{2\alpha}$ at mid cycle was similar to the response found when this same treatment was administered in late cycle.

The results of this study would indicate that the injection of gonodotrophins for the induction of multiple births can be timed from an induced estrus using $PGF_{2\alpha}$, thereby reducing some of the labor required for the induction of multiple births. However, the injection of $PGF_{2\alpha}$ after PMSG does not appear to reduce the ovulation rate compared to when PMSG is given alone. This would indicate that multiple birth production will not become practical until a method is developed for controlling the ovulation rate.

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APPENDIX

TABLES

TABLE VI

STOCK BUFFER SOLUTIONS USED IN IMMUNOASSAY PROCEDURES

1. 0.5 Sodium Phosphate Buffer, pH 7.5

   A) Weigh 69.0 g $Na_2PO_4 \cdot H_2O$ (monobasic) and dilute to 1000 ml with glass distilled H_2O; store at 5°C.

   B) Weigh 71.0 g Na_2HPO_4 (dibasic) and dilute to 1000 ml with glass distilled H_2O; store at 5°C.

   C) To make 0.5 M Sodium Phosphate, mix 1 part monobasic plus 4 parts dibasic. Adjust pH to 7.5.

2. Stock Phosphate Buffered Saline

   A) 120 ml of 0.5 M Sodium Phosphate, $NaH_2PO_4 \cdot H_2O$ (monobasic)

   B) 240 ml of 0.5 M Sodium Phosphate, $NaHPO_4$ (dibasic)

   C) 143 g Sodium Chloride, NaCl

   D) 1.75 g Thimerosol (Merthiolate)

   E) Add glass distilled H_2O to a final volume of 3500 ml

   F) Check pH, adjust if necessary.

TABLE VII

WORKING BUFFER SOLUTIONS USED IN IMMUNOASSAY PROCEDURES

1. Phosphate Buffered Saline Working Solution (PBS)

   A) Dilute one part PBS Stock with four parts glass distilled water

2. Phosphate Buffered Saline Plus 0.1% Gelatin (PBS + Gel)

   A) Weigh 1 g Knox Gelation and dilute to 1000 ml with PBS Working Solution.

TABLE VIII

LIQUID SCINTILLATION FLUIDS

1. Steroid Counting Fluid

   A) Weigh 15 g PPO

   B) Weigh 0.15 g POPOP

   C) Combine PPO and POPOP with 3800 ml Scintillation grade Toluene and mix until dissolved.

2. Protein Binding Counting Fluid

   A) Weigh 21.7 g PPO

   B) Combine PPO with 3000 ml Scintillation Grade Toluene and mix until dissolved.

TABLE IX

PLASMA PROGESTERONE IN ANIMALS EXHIBITING ESTRUS FOLLOWING TREATMENT IN MID CYCLE

Day Post PGF-I	Trt. I Control (ng/ml)	Trt. II PMSG Day 12 PGF Day 13	Trt. III PGF Day 13 (ng/ml)
0	7.7±1.8*	9.8±1.1	7.8±0.6
3	0.8±0.0	1.0±0.1	1.0±0.1
12	5.8±0.5	4.9±0.8	8.2±1.1
13	---	8.5±2.0	10.0±1.2
14	7.4±1.1	1.9±0.3	3.6±0.6
15	---	1.2±0.2	1.4±0.3
16	8.2±1.3	1.0±0.1	0.7±0.1
17	---	1.3±0.2	0.7±0.1
18	8.8±0.9	---	0.5±0.0

*Mean ± S.E.

TABLE X

PLASMA PROGESTERONE IN ANIMALS EXHIBITING ESTRUS FOLLOWING TREATMENT IN LATE CYCLE

Day Post PGF-I	Trt. I Control (ng/ml)	Trt. IV PMSG Day 20 (ng/ml)	Trt. V PMSG Day 20 PGF Day 21
0	7.7±1.8*	7.7±1.1	7.8±1.2
3	0.8±0.0	1.0±0.2	1.0±0.2
12	5.8±0.5	6.5±0.8	6.6±0.9
14	7.4±1.1	7.1±0.5	7.4±0.7
16	8.2±1.3	8.5±1.0	8.5±0.8
18	8.8±0.9	7.2±0.5	8.4±0.5
20	8.2±1.7	8.0±0.8	10.2±0.7
21	---	12.6±0.8	12.1±0.6
22	7.5±2.0	10.8±2.3	2.6±0.4
23	5.0±1.5	8.5±2.1	1.5±0.4
24	3.9±2.3	4.9±1.9	1.2±0.5
25	3.3±2.4	3.9±2.5	1.8±0.6
26	1.6±0.8	1.7±0.7	2.5±0.0
27	1.6±0.0	0.8±0.0	---

*Mean ± S.E.

TABLE XI

PLASMA ESTRADIOL IN ANIMALS EXHIBITING ESTRUS
FOLLOWING TREATMENT IN MID CYCLE

Day Post PGF-I	Trt. I Control (pg/ml)	Trt. II PMSG Day 12 PGF Day 13	Trt. III PGF Day 13 (pg/ml)
0	2.5±0.4*	4.7±0.2	4.9±0.7
3	7.5±0.8	7.4±0.9	9.9±2.2
12	3.1±0.5	5.5±0.2	5.3±0.7
13	---	9.8±2.7	6.4±0.8
14	3.4±0.3	16.0±5.5	7.7±1.3
15	---	19.0±8.7	9.0±1.0
16	3.9±0.9	17.2±2.7	11.7±1.1
17	---	20.5±0.4	15.7±2.4
18	4.0±0.6	---	8.9±0.0

*Mean ± S.E.

TABLE XII

PLASMA ESTRADIOL IN ANIMALS EXHIBITING ESTRUS
FOLLOWING TREATMENT IN LATE CYCLE

Day Post PGF-I	Trt. I Control (pg/ml)	Trt. IV PMSG Day 20 (pg/ml)	Trt. V PMSG Day 20 PGF Day 21
0	2.5±0.4*	4.0±0.6	2.6±0.5
3	7.5±0.8	5.3±0.6	5.7±0.4
12	3.1±0.5	3.9±1.0	3.5±0.6
14	3.4±0.3	3.2±0.5	4.0±0.1
16	3.9±0.9	5.8±1.1	4.4±0.7
18	4.0±0.6	6.2±0.9	4.4±0.6
20	3.1±0.4	5.5±0.7	3.9±0.5
21	---	9.3±2.7	8.0±0.9
22	3.9±0.4	22.1±10.2	15.9±6.8
23	7.9±1.2	32.7±9.0	17.6±5.5
24	6.9±1.6	43.8±16.6	8.7±2.5
25	7.2±2.4	51.3±33.7	11.0±2.4
26	5.9±0.2	56.4±36.5	5.1±2.2
27	6.6±0.0	10.5±0.0	11.4±0.0

*Mean ± S.E.

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